THE WATER CONTENT OF THE STRATUM CORNEUM

III. Effect of Previous Contact with Aqueous Solutions of Soaps and Detergents*

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The major barrier against penetration of various substances into the skin is located near the base of the stratum corneum. Therefore substances placed on the surface of the intact skin can penetrate into the stratum corneum more easily than they can reach tissues below the barrier. Substances applied to the outer surface of the skin may act upon that portion of the skin outside the barrier, upon the barrier itself, upon that portion of the skin beneath the barrier, or upon all of these. In this paper will be discussed the action of chemicals used in the manufacture of various household soaps and detergents on that portion of skin which lies outside the barrier.

Frequent contact with soaps and detergents is thought to contribute to dryness of the skin. The first signs of dryness of the skin are scaling and lack of flexibility of the cornified epithelium (1). Flexibility of the cornified epithelium can be shown to be a function of its water content (2). If the cornified epithelium is flexed after it has lost water and has become brittle, small breaks will occur which result in scaling and roughness of the surface.

The cornified epithelium is hydrophilic and attracts water from a humid environment, thus maintaining its flexibility. Pretreatment of pieces of cornified epithelium with some organic solvents so alters this tissue that water can subsequently dissolve up to 20 per cent of the callus and the extracted callus can no longer absorb as much water from the environment (3). Since it holds less water, it is also less flexible. The question has arisen as to whether or not solutions of household soaps and detergents dissolve hydrophilic materials from the cornified epithelium, thus reducing its ability to absorb water from the environment and leaving it brittle. If this does occur, this mechanism alone might explain the mild dryness which may result from frequent contact with soap and detergent solutions.

Severe dryness and chapping undoubtedly have a more complex pathogenesis. The inflammation accompanying this condition no doubt indicates that substances have passed into the tissues below the barrier, either by penetration of the the barrier itself or via breaks in the barrier.

MATERIALS

The materials† used in this study consisted of an alkaline phosphate, which is commonly used as a builder in household soaps and detergents, a soap, and

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† These materials were supplied by The Procter & Gamble Company, Cincinnati, Ohio

two synthetic detergents. Descriptions of these materials, and the codes by which they will be identified throughout this paper follow:

1. STP is sodium triphosphate, technical grade, containing about 90 per cent sodium triphosphate ("tripolyphosphate," $Na_5P_3O_{10}$) and 10 per cent sodium pyrophosphate ($Na_4P_2O_7$).

2. CNAS is an alkyl sulfate derived from coconut oil by reduction to fatty alcohol, sulfation, and neutralization. It contains some moisture (0.5–1.3 per cent) and some sodium sulfate in addition to the alkyl sulfate (91 to 95 per cent). The distribution of chain lengths is similar to that for a coconut oil soap.

3. ABS is an alkylbenzene sulfonate, with the alkyl group largely dodecyl (tetrapropylene). It is between 90 and 96 per cent pure, the impurities being water (about 2 per cent), unsulfonated alkylbenzene (about 1 per cent), sodium sulfate, and sodium chloride.

4. SOAP is a coconut oil soap, containing the sodium salts of the fatty acids occurring naturally in coconut oil, with sodium laurate predominating. It is about 95 per cent pure soap, the remainder being mostly water and a small amount of inorganic salt.

THE EFFECT OF SOAP AND DETERGENT SOLUTIONS ON THE WATER-HOLDING CAPACITY OF CORNIFIED EPITHELIUM

Calluses which had been removed as single pieces from the plantar surface of the foot were sanded to a uniform thickness of 0.3 mm. Each callus was hung in a weighing bottle over concentrated sulfuric acid for two weeks in order to obtain an original dry weight, then hung successively in weighing bottles over 30, 20, and 10 per cent sulfuric acid solutions. These solutions at 23°C. maintain environments of 76, 88, and 97 per cent relative humidity respectively. Equilibrium is reached in the environments of 76 and 88 per cent relative humidity in 24 hours. At 97 per cent relative humidity, the calluses were allowed to remain for 48 hours. The increase in weight in each environment was determined. From these data the pretreatment curve for the water-holding capacity of each callus could be determined.

Each callus was then placed in 25 ml. of either water or a 1 per cent solution of one of the various materials for 2 hours at 40°C. When mixtures were used, each material was present in 1 per cent concentration. Following this treatment, each callus was transferred to 200 ml. of water at 40°C. for 30 minutes in order to wash out any excess free soap or detergent. When washing was complete, the calluses were again hung over concentrated sulfuric acid for two weeks so that a new dry weight might be obtained. Finally, the increase in weight in environments of 76, 88, and 97 per cent relative humidity was determined in the same way as before. The water-holding capacity of the calluses after treatment is calculated on the dry weight after treatment.

The sheets of callus which were soaked in water alone subsequently show only a slight decrease in their ability to absorb water from the environment. This is also true for callus soaked the same length of time in 1 per cent sodium triphosphate. After soaking in 1 per cent soap or detergent solutions, the calluses are able to hold somewhat less water than before treatment with these solutions, particularly at high relative humidities. The synthetic detergent solutions do not alter the water-holding capacity any more than does the soap solution. Addition of 1 per cent sodium triphosphate to the soap or detergent solutions causes about the same alteration in the water-holding capacity of calluses as do the soap and detergent solutions alone. Representative curves for the water-holding capacity of sheets of callus before and after soaking in water and the various solutions are shown in Fig. I.

We know of no satisfactory way of measuring the flexibility of the sheets of callus objectively. They were always felt for flexibility after coming to equilibrium in the various environments. After observations on many calluses, we feel that it is fair to say that at each relative humidity the flexibility of the calluses is decreased following a two-hour immersion in water, even though the water-holding capacity is not decreased. Immersion in either a soap or detergent solution decreases the flexibility even more than immersion in water, but no major difference in flexibility was observed between calluses soaked in soap solution and those soaked in detergent solutions.

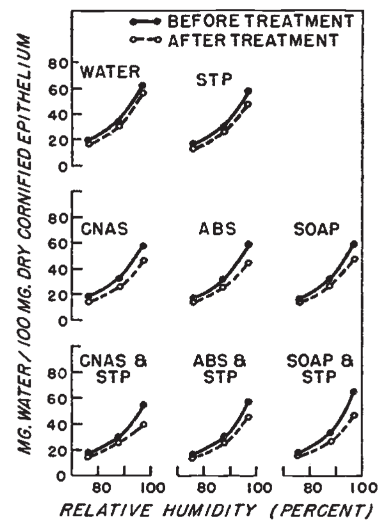

FIG. I. Showing the water-holding capacity of calluses at various relative humidities. See text for details of treatment.

Thus, it appears that cornified epithelium will lose some of its flexibility when soaked in water, or in soap solutions, or in solutions of synthetic detergents. This loss of flexibility cannot be explained entirely on the basis of loss in water-holding capacity, since soaking in water alone does not alter the water-holding capacity very much, but does alter the flexibility. Soaking in soap or detergent solutions alters both water-holding capacity and flexibility. Possibly the extraction of various materials from the cornified epithelium by water and aqueous solutions of soaps and detergents is responsible for the change in flexibility and/or the water-holding capacity of this tissue.

EXTRACTION OF NITROGENOUS MATERIALS FROM CALLUSES BY WATER AND AQUEOUS SOLUTIONS OF SOAPS AND DETERGENTS

If a thin sheet of callus is soaked in water, the total amount of extracted solids can be determined simply by evaporating off the water and weighing the residue.

If aqueous solutions of soaps or detergents are used, however, the residue will contain not only the extractives, but also the dry soap or detergent. The weight of dry solute will be large compared to the weight of extractives. A small and not easily determinable amount of the solute will be held by the callus. Therefore there is no simple way of measuring the total weight of solids extracted from calluses by solutions of soaps and detergents. Since probably a major portion of the extractives are nitrogenous materials and since the soaps and detergents used contain no nitrogen, it was decided to estimate the amount of extractives by determining the total nitrogen in the solutions.

The nitrogenous substances come from the cornified epithelium itself and from substances which have been excreted onto the surface of the skin and reabsorbed into the callus (4). Among the nitrogenous materials are the aromatic amino

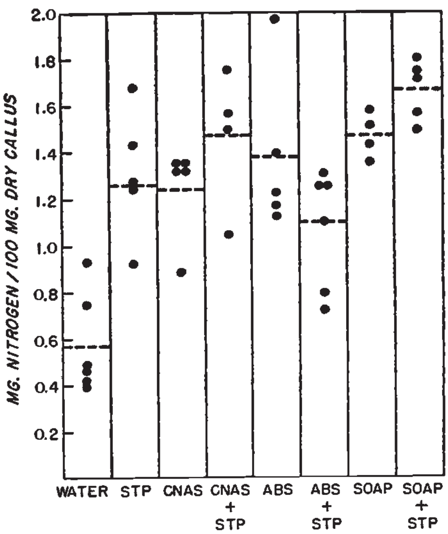

FIG. II. Showing the amount of nitrogenous material extracted from thin sheets of callus by water and by 1 per cent aqueous solutions of the substances indicated. Dotted lines show the averages of the points.

acids or combinations of these amino acids in the form of peptides. These aromatic amino acids show a peak in their absorption spectrum between 275 and 280 mμ.

The water and solutions in which the calluses had been immersed for 2 hours at 40°C., as previously described, were filtered. The Pregl modification of the Kjeldahl method was used to determine the total nitrogen. Caprylic alcohol was added to the solutions during digestion to prevent excessive foaming.

For determining the ultraviolet absorption spectrum, 1 ml. of each solution was diluted with 4 ml. of a 1 per cent solution of sodium triphosphate (STP). This adjusted the pH of all solutions to 9.0 ± 0.1. Similar dilutions were made from one-milliliter samples of the original solutions to serve as blanks. A Beckman spectrophotometer, Model DU, with ultraviolet attachment, was used for these determinations.

Figure II shows the amount of nitrogenous material per 100 mg. dry callus which is extracted by immersion of the calluses in water and various aqueous solutions for 2 hours at 40°C. It is seen at once that there is a wide variation from callus to callus. This certainly might be expected since the history of any one piece of callus previous to the time it was removed from the foot is indeterminable. The amount of washing the foot had received is unknown. If the foot had been washed frequently, less of the nitrogenous materials excreted in the sweat would have accumulated in the callus and more of the water-soluble portions of the cornified epithelium would have been removed.

From Fig. II it can be seen that each of the aqueous solutions extracts more nitrogenous material than does water alone. The soap solution extracts as much nitrogenous material as do the solutions of the synthetic detergents. The sodium triphosphate solution extracts more than does water and the addition of this alkali to either the SOAP or the CNAS solutions may slightly increase the amount of extractives. The addition of STP to the ABS solution may have decreased the

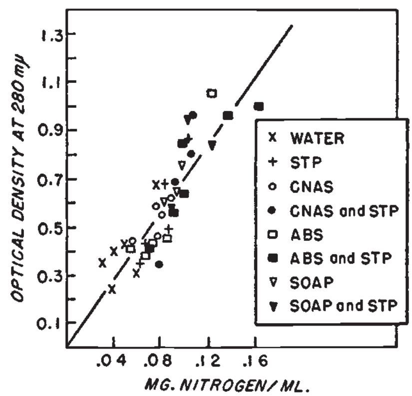

FIG. III. Showing the relationship of the optical density to the nitrogen content of solutions of soap and detergents in which calluses have been soaked.

nitrogenous extractives, but with such a wide range of variability this may not represent a true decrease.

In Figure III, the optical density at 280 mμ of the solutions obtained after extracting the calluses is plotted against the nitrogen content of these solutions. Again, there is considerable variation for different calluses. For the solutions of soap and each detergent, however, the points scatter more or less uniformly to both sides of a single straight line, indicating a constant percentage of aromatic amino acids in the extractives independent of which chemical is used in the extracting solution.

EXTRACTION OF NITROGENOUS MATERIALS FROM INTACT, LIVING SKIN BY WATER AND AQUEOUS SOLUTIONS OF SOAPS AND DETERGENTS

When water or aqueous solutions are in contact with the living skin, water-soluble extractives are removed from the cornified epithelium just as these materials are removed from calluses. It is unlikely that any major portion of the nitrogenous extractives is dissolved from the lower layers of normal skin since such substances would have to pass through the barrier at the base of the cornified epithelium and probably most of these nitrogenous substances are of such a molecular size that passage through the barrier would be very slow.

Conceivably, some of the nitrogenous material in water which has been in contact with living skin for some time might come from active sweating during the contact period. Shelley (5) showed, however, that swelling of the cornified epithelium as a result of wet dressings causes poral closure and blockage of sweat delivery to the surface of the skin. Our microscopic observation of the finger tips seemed to indicate that blockage of the sweat ducts occurred during the first five minutes of immersion of the hand in aqueous solutions. Therefore, when aqueous solutions are held in contact with intact, living skin, it might be

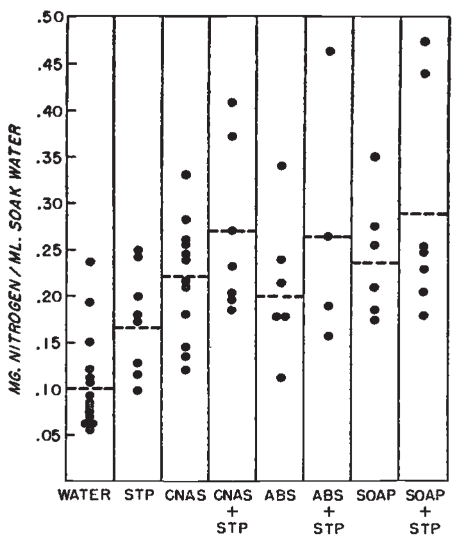

FIG. IV. Showing the amount of nitrogenous material extracted from the skin of the hands by water and by 1 per cent aqueous solutions of the substances indicated. Dotted lines show the averages of the points.

expected that the extractives obtained would be similar to those found in solutions in which calluses had been immersed.

Rubber gloves were rinsed at least five times in distilled water. After placing the gloves on the subject's hands, 50 ml. of water or of 1 per cent solution of the detergents or soap was added to each glove. The solutions remained in contact with the skin for 30 minutes, and were then filtered. Nitrogen content of the solutions and the optical density at 280 mμ were determined as outlined above, except that for the optical density measurements 1 ml. of a solution was diluted with 9 ml. of 1 per cent STP.

Just as with the data obtained from calluses, Figure IV shows a wide range of variability in the amount of nitrogenous extractives in the various solutions. These data were obtained from seven subjects. No effort was made to hold constant the interval between the experimental procedure and a previous washing of the hands. Repeat determinations on one subject showed less variation than determinations from subject to subject.

As with the calluses, the soap solution and the solutions of the synthetic detergents extracted more nitrogenous materials than did water. No major variation is seen between the soap and the synthetic detergent extractives. Sodium triphosphate extracted more nitrogenous materials than did water, and the addition of this alkali to each of the detergent solutions caused increased extraction.

When the optical density at 280 mμ of the solutions from the hand-soaks is plotted against the mg. nitrogen per ml. of these solutions, there is considerable scatter of the points (Figure V). As with similar data from the calluses, however, there is indication that for each of the solutions the aromatic amino acids make up a relatively constant percentage of the total nitrogenous material. All points tend to scatter about equally on either side of a single straight line.

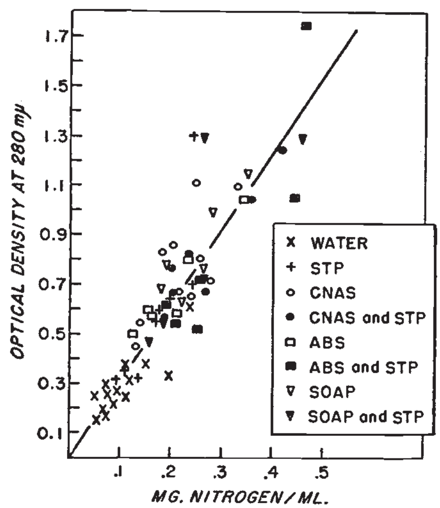

FIG. V. Showing the relationship of the optical density to the nitrogen content of solutions of soap and detergents in which the hands have been soaked.

SUMMARY AND CONCLUSIONS

After soaking in water or in aqueous solutions of soaps or synthetic detergents, thin sheets of callus are less flexible than before soaking. Soaking in water does not reduce the water-holding capacity of cornified epithelium very much. Soaking in soap solutions reduces the water-holding capacity as much as does soaking in synthetic detergent solutions. The reduction of the water-holding capacity is not measurably altered by the addition of sodium triphosphate to either the soap or the synthetic detergent solutions.

There are water-soluble nitrogenous materials which can be extracted from calluses or from intact cornified epithelium by water alone or by aqueous solutions of soap or synthetic detergents. A soap solution extracts as much of these materials as do synthetic detergent solutions. In all of these extractives there appears to be a relatively constant amount of aromatic amino acids.

There exists a clinical impression that the routine use of commercial household synthetic detergents is more damaging to the skin than is the routine use of household soaps. The data reported here do not indicate that the chemicals commonly used in the manufacture of household detergents alter cornified epithelium any differently than does a coconut oil soap. If synthetic detergents are more damaging to the skin than soap, the mechanism of this action has not been determined by the investigations on cornified epithelium reported in this paper.

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DISCUSSION

Dr. Stephen Rothman (*Chicago, Ill.*): The great significance of Dr. Blank's research needs not to be emphasized before this audience. His was a classically simple experiment when he has shown that pliability of a dried callus can be restored by water but not by oil. It appears that the normal horny layer requires a certain amount of hydration to maintain its flexibility. Dr. Blank did not claim that the lipid film does not play a role in maintaining the flexibility of the stratum corneum but he interprets the role of lipids as hindering to some degree the evaporation of water from the horny layer. It seems that the health of the skin requires a certain degree of hydration in the horny layer. If the horny layer is overhydrated ("macerated") there is an increased susceptibility to bacterial and fungal (particularly monilial) infection. Pillsbury and his group have conclusively shown that defense against bacterial infection depends primarily on dryness of the horny layer. On the other hand, if the horny layer becomes excessively dry, chapping and cracking ensues and in the end this xerosis leads to inflammatory reactions. Sulzberger has emphasized that one hardly ever sees chapping in summer, even not if the air is extremely dry and soaps and detergents are used liberally, while in fall and winter chapping develops with great ease. Apparently there is greater hydration of the horny layer in summer than in winter. I would like to ask Dr. Blank to comment on this phenomenon. It probably would be important to establish numerically the desirable degree of hydration of the horny layer, particularly on the dorsal and palmar surfaces of the hands, and to find out how much is too much and how much is too little.

Dr. Marion B. Sulzberger (*New York, N. Y.*): I agree heartily with the laudatory comments of Dr. Rothman concerning the very beautiful experiments of Dr. Blank. We all know them and recognize their great fundamental significance; and I need not repeat what Rothman has said so well about the various directions in which they are significant. I would like to make just one comment in reference also to Dr. Rothman's discussion. Dr. Elfriede Ehrenreich and I came upon this phenomenon (which I am sure everyone knew clinically) but which we first saw experimentally when we were commissioned by a large pharmaceutical and cosmetic house to try to study various formulations of "hand lotions" on human skin in order to ascertain which would best prevent "chapping." Various formulas which the Company's chemists had elaborated were to be tried out and compared. In order to do this we needed a series of "chapped" hands readily available, and to obtain these we tried to devise a means of making hands "chap." The company offered the employees of their large plant in the vicinity of New York, the lady employees, who served as volunteers and who would be paid for allowing their hands to be "chapped." The striking, and to us at that time surprising, finding was that we could not produce chapping in these employees during the summer months, no matter what we did to them. For example, we let them immerse their hands in all kinds of detergents, in soapy water at various temperatures; immediately afterwards we had them put their wet hands into refrigerated spaces with fans going, in order to try to imitate the effects of the degreasing, the cold, wind, etc—all to no avail! Whereas as soon as the fall came and cold weather began, we produced "chapping" of the hands regularly and with great ease. I think that perhaps one explanation—and I would like to hear Dr. Blank's opinion of the correctness of this explanation—is the following: the lipid film on the skin surface must not be considered as merely a fatty layer which prevents the evaporation of water but actually as acting as an emulsifying agent; and at the same time as a lipid moiety of a labile emulsion which presents a reservoir from which the horny layer (keratin layer) and the skin surface can extract and take up water when it needs it. In other words, sebum, sweat, insensible perspiration, secretory perspiration and horny meshwork—all put together, form a receptacle, a lipid emulsion of water in oil and sometimes oil in water on the skin surface; and the watery phase of this presents a source whence the horny layer can get water for its required hydration and softening. This watery reservoir is greater in warm, humid weather, leads to more keratin hydration, as well as to more flow and spread of oil and lipids over the surface and into the interstices—and thereby tends strongly to prevent or reduce "chapping" during the summer in New York City.

Dr. Samuel M. Peck (*New York, N. Y.*): We had the problem of trying to reproduce chapping in winter or summer conditions, in studying a hand lotion in preventing chapping. We tried the same procedure as Dr. Sulzberger using detergents with immersion for long periods of time in water with uniformly bad results, because we could not reproduce the chapping consistently if at all.

We worked out a very practical method of producing chapping winter or summer, just along the lines that you have pointed out. We used a dry ice sludge with acetone and then you could produce chapping very easily in any degree you wished, depending on the duration and number of applications.

Dr. Franz Herrmann (*New York, N. Y.*): This is a fundamental contribution to our knowledge of the hydration capacity of the stratum corneum. I should like to ask whether lipid analyses of the callus specimens were performed, in parallel with the other observations prior to—and after treatment with the aqueous test solutions; and whether or not sweat was tried as a test solution.

DR. DAPHNE ANDERSON (*Philadelphia, Pa.*): Just one point: I should like to ask Dr. Blank a question of the actual technic in regard to the aromatic amino acids. It would be very true that you would get some of these amino acids in sweat and also there are possibly other substances in sweat which might give similar curves on the Beckman. I wondered whether one would be right in the present circumstances to judge that the aromatic amino acids obtained in the extracts were washed out from the stratum corneum or produced by sweat contamination.

DR. IRVIN H. BLANK (*in closing*): It certainly is quite understandable that several of the discussers would bring up the role of lipids or sebum in maintaining a soft cornified epithelium. I think there is one misconception about some of our previous work as well as that of Burch. I do not think we have ever stressed the role of the lipid film on the surface of the skin as being that of a barrier against the evaporation of water from the cornified epithelium. Burch was the first to show that he was unable to alter the rate of water loss through the skin as a result of removing this film from the surface of the skin. We repeated that work and did not show any major alteration in the rate of water loss when the surface film of lipids was removed. This would throw some doubt as to the importance of the lipid film in helping prevent the evaporation of water from the cornified epithelium. However, this does not tell us just what role sebum does play in maintaining a softened cornified epithelium.

Dr. Sulzberger suggests that sebum helps emulsify water in the neighborhood of the stratum corneum and thus serves as a reservoir for subsequent absorption of water by the stratum corneum. Perhaps this is correct. Hydrophilic materials such as glycerin seem to be able to attract water from the environment and make it available to the stratum corneum. Perhaps in some similar way the sebum can hold water in reserve for the stratum corneum.

Actually, it is my opinion that at the present time data which will permit us to state the exact role of sebum in maintaining a soft and flexible stratum corneum are not yet available.

Dr. Peck, did you actually immerse the hands in the slush of acetone and dry ice?

DR. PECK: We rubbed the skin with this slush.

DR. BLANK: This sounds like drastic treatment. I do not know why it is so difficult to produce chapping in the summer time. There has been some attempt to produce chapping during the summer by holding the hands in an atmosphere of very low relative humidity. According to our thesis, this ought to produce chapping. We have not done this experiment ourselves, but have been told by those who have tried it that the skin does not chap. Blood circulation possibly plays a role here, but I do not know what role it plays. Chapping is probably considerably more complex than the simple alteration of the water content of the cornified epithelium.

Dr. Herrmann, we did not determine the lipid content of our extracts nor did we treat the calluses with sweat; so I have no answer to either of the questions you raised.

Dr. Anderson raised a question about the source of aromatic amino acids. I did not discuss this during my presentation, but it is discussed in the printed paper. It is, of course, correct, as she states, that the aromatic amino acids which appear in extracts of callus may have been deposited in the callus by the sweat or they may come from the decomposition of proteins in the cornified epithelium. A third source is possible in the aqueous solutions which were in contact with living skin. The amino acids could come from active sweating during the time the solutions were in contact with the skin. We believe that there is very little active sweating into the solutions.